

University of Groningen

## Effects of light exposure and sleep displacement on dim light melatonin onset

Gordijn, M C M; Beersma, D G M; Korte, H J; van den Hoofdakker, R H

*Published in:*  
Journal of Sleep Research

*DOI:*  
[10.1046/j.1365-2869.1999.00156.x](https://doi.org/10.1046/j.1365-2869.1999.00156.x)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1999

[Link to publication in University of Groningen/UMCG research database](#)

### *Citation for published version (APA):*

Gordijn, M. C. M., Beersma, D. G. M., Korte, H. J., & van den Hoofdakker, R. H. (1999). Effects of light exposure and sleep displacement on dim light melatonin onset. *Journal of Sleep Research*, 8(3), 163 - 174. <https://doi.org/10.1046/j.1365-2869.1999.00156.x>

### **Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### **Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Effects of light exposure and sleep displacement on dim light melatonin onset

M. C. M. GORDIJN, D. G. M. BEERSMA, H. J. KORTE and  
R. H. VAN DEN HOOFDAKKER

Department of Biological Psychiatry, University Clinic, Groningen, The Netherlands

Accepted in revised form 27 January 1999; received 2 June 1998

**SUMMARY** The purpose of the study was to induce in two different ways, a phase-angle difference between the circadian pacemaker and the imposed sleep–wake cycle in humans, we intended to: (i) shift the circadian pacemaker by exposure to bright light and keep the timing of the sleep–wake cycle fixed; and (ii) keep the timing of the circadian pacemaker fixed by a constant light–dark cycle and displace sleep. We monitored dim light melatonin onset (DLMO), core body temperature and sleep. DLMO was delayed significantly after 3 days of a 3-h delayed sleep-phase when compared with 3 days of sleep at a normal or 3-h advanced sleep-phase. The shifts in DLMO were not accompanied by shifts in body temperature, changes in waking-up time or by a change in the duration of the first rapid eye movement (REM) sleep episode. Three days of light exposure in the morning or evening resulted in shifts in DLMO of similar magnitude, but this was accompanied by shifts in the rhythm of body temperature, changes in waking-up time and in the duration of the first REM sleep episode. We conclude that the changes observed after light exposure reflect shifts in the circadian pacemaker. In contrast, we propose that the changes observed in DLMO after sleep displacement are not mediated by the circadian pacemaker. These results raise some doubts about the reliability of DLMO as a marker of circadian phase in cases of sleep disturbances. Finally, we initiate a search for changes in sleep that might be responsible for the unexpected effects on DLMO.

**KEYWORDS** melatonin, sleep displacement, light exposure, circadian pacemaker, phase shift

## INTRODUCTION

Theories of chronobiological disturbances underlying sleep disturbances or mood disorders have frequently been the topic of discussion (e.g. Van den Hoofdakker 1994; Terman 1995; Wirz-Justice 1995). We conducted two experiments in an attempt to contribute to the discussion on the possible role of chronobiological processes in mood regulation. The purpose of these experiments was to induce, in two different ways, phase-angle differences between the circadian pacemaker and the imposed sleep–wake cycle, and to record their effects on mood. In the first study, our intention was to shift the circadian

pacemaker, by exposing subjects to bright light, and keep the timing of the sleep–wake cycle fixed. In the second study we intended to keep the timing of the circadian pacemaker fixed, by imposing a constant light–dark cycle, and to displace sleep.

Some of the results of the first experiment, a cross-over study of bright-light exposure in the morning and evening in depressed and healthy subjects, have been presented previously (Gordijn *et al.* 1998). Exposure to bright light did, in fact, induce phase shifts of several variables that were thought to be under circadian control, without clear effects on mood. We used dim light melatonin onset (DLMO), the rhythm of core body temperature and sleep termination as markers of circadian phase.

The results of the second study, a cross-over study of an advanced and delayed sleep phase with a fixed light–dark cycle in healthy subjects, have not been presented before. In this

*Correspondence:* Dr M. C. M. Gordijn, Zoological Laboratory, University of Groningen, PO Box 14, 9750 AA, Haren, The Netherlands. Tel.: +31 50 363 7658; fax: +31 50 363 2148; e-mail: m.c.m.gordijn@biol.rug.nl.

latter study, we also used DLMO as one of the markers of the phase of the circadian pacemaker. To our surprise, we found shifts in DLMO after a delay of the sleep phase – a condition that we had not expected to affect the circadian pacemaker. Therefore, we present the two experiments in this paper and compare the effects of exposure to light and sleep displacement on DLMO, core body temperature and sleep. Finally, we initiate a discussion on the possible mechanisms causing the phase shifting effect of sleep displacement on DLMO.

DLMO, i.e. the time when the melatonin concentration starts rising in the evening, is one of the variables that was recorded to assess the phase of the circadian pacemaker. The 24-h rhythm of the secretion of melatonin by the pineal gland is thought to be controlled directly by the circadian pacemaker (for a review see Arendt 1988) and DLMO is thought to be a reliable phase marker (Lewy *et al.* 1985). So far, only a small number of factors that have an acute effect on the melatonin concentrations in blood or saliva have been identified. The most important factors we know of are retinal light exposure, (e.g. Lewy *et al.* 1980; Trinder *et al.* 1996), nonionizing radiation with a frequency range under 60 Hz (Reiter and Richardson 1992), posture (Deacon and Arendt 1994) and activity (Carr *et al.* 1981; Monteleone *et al.* 1990). In addition, contamination of saliva can affect the radioimmunoassay (RIA) in such a way that the outcome becomes unreliable (Gordijn *et al.* 1991).

So far, there has been a general agreement that the phase-shifting effects of bright light on the rhythm of melatonin secretion, and on DLMO in particular, can be assessed in a reliable way if all these factors are controlled (e.g. Lewy *et al.* 1985; Dijk *et al.* 1989). These shifts are interpreted as reflecting phase shifts of the circadian pacemaker. The use of DLMO has therefore been recommended as a method of assessing the phase of the circadian pacemaker ('phase typing') in 'patients suspected of having a chronobiologic component to their sleep or mood disorder' (Lewy and Sack 1987). It is claimed that the abnormal timing of DLMO provides an indication for the optimal timing of treatment, i.e. exposure to light, in these patients.

In addition to DLMO, we recorded core body temperature as an indicator of circadian phase and sleep parameters that are known to be (partly) under circadian pacemaker control, in this case 'sleep timing' and the occurrence of rapid eye movement (REM) sleep.

Core body temperature is the marker commonly used for circadian phase in humans. A phase-response curve has been described for the effects of light exposure on the rhythm of body temperature (Honma and Honma 1988; Minors *et al.* 1991).

Parameters of the sleep-wake cycle itself are used less frequently as phase markers in humans, although it has been shown that sleep duration is highly dependent on circadian phase (Czeisler *et al.* 1980; Dijk and Czeisler 1995) and a phase-response curve for the sleep rhythm to bright light has been derived (Honma and Honma 1988). The theoretical background for the circadian control of sleep timing is elaborated in the two-process model of sleep regulation

(Borbély 1982; Daan *et al.* 1984). This model predicts that the timing of sleep is determined by the interaction of a homeostatic 'sleep need' and a circadian process, that controls an upper and lower threshold for this homeostatic process. If the prior sleep-wake cycle is known, the model predicts sleep timing after manipulations of the circadian pacemaker. Dijk *et al.* (1987) confirmed this. They reported earlier sleep termination after morning light exposure.

Other sleep parameters besides sleep timing are influenced to different degrees by circadian processes. The occurrence of REM sleep, for instance, is thought to depend partly on circadian phase and partly on the sleep process itself (Czeisler *et al.* 1980; Dijk and Czeisler 1995). Changes in the duration of the first REM sleep episode have been interpreted either as changes in the phase of the circadian pacemaker or as direct results of phase shifts of core body temperature (Cajochen *et al.* 1997).

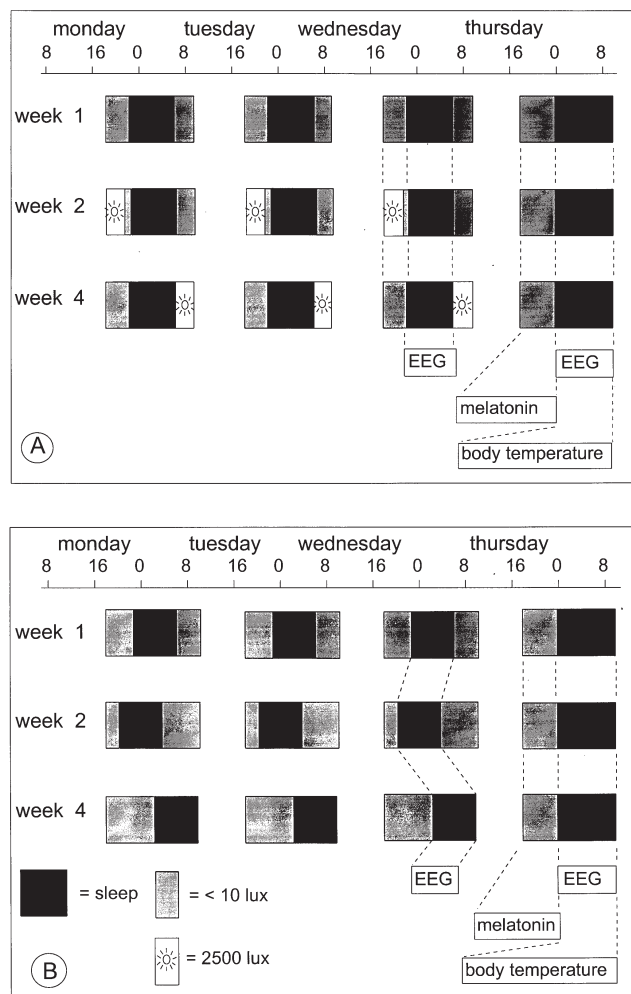
## METHODS

### Design study 1, light exposure

The design of study 1 (Fig. 1a) has been described previously in Gordijn *et al.* (1998). In the latter study, eight depressed patients and eight healthy controls participated in an experiment, the purpose of which was to induce phase shifts of the circadian pacemaker by light exposure. The effects of the light exposure on circadian rhythms, sleep and subjective mood ratings were analysed. In the present study, the effects on the circadian rhythms and sleep of seven of eight healthy subjects from that study were included. Subject 8 was excluded because of one missing sleep EEG recording. Subsequently, five new subjects were added to the group.

The experimental period lasted 4 weeks. During the first, second and fourth week (henceforth referred to as experimental weeks) all subjects stayed in the laboratory from 18.00 to 09.00 the next morning, on three consecutive days. In the third week no laboratory visits were scheduled. In the experimental weeks, subjects followed their normal daily routine between 09.00 and 18.00. The intensive pursuit of sports, drinking of alcoholic beverages and sleeping in the daytime were not allowed. There were no restrictions during the daytime with respect to coffee intake. In the laboratory, the subjects stayed in dim light (< 10 lux) in separate rooms. During the first three nights of each experimental week, time in bed was fixed from 22.00 to 06.00 (0 lux). During the fourth evening, subjects arrived at the laboratory at 18.00 and stayed in dim light from that time on until 00.00. At 00.00, they went to bed and were instructed not to get up until they felt refreshed.

During the first three evenings and mornings of the experimental weeks, the levels of light exposure were varied. The first week with dim light (< 10 lux) exposure in the morning and evening (DL) served as control. During the second week subjects were exposed to bright light (2500 lux) on three consecutive days, six of them in the morning from 06.00 to 09.00 (ML) and six in the evening from 18.00 to 21.00 (EL). In the fourth week the conditions were reversed.



**Figure 1.** Design of study 1 (A, light exposure) and study 2 (B, sleep displacement)

### Design study 2, sleep displacement

The design of study 2 (Fig. 1b) was similar in duration and procedure to that of study 1. The purpose of this second study was to shift the time when sleep was allowed without inducing a change in the light–dark cycle.

During the experimental weeks of a 4-week period (weeks 1, 2 and 4), the subjects stayed in the laboratory on three consecutive nights from 18.00 to 10.00 the next morning. During the first week they were allowed to sleep on these three nights from 23.00 to 07.00 (NS, normal sleep, 0 lux). They spent the remaining time (18.00–23.00 and 07.00–10.00) in dim light (< 10 lux) in separate rooms. During the first three nights of the second and fourth week, the time they were allowed to sleep was either shifted 3 h in advance (ES, early sleep from 20.00 to 04.00, 0 lux, and dim light from 18.00 to 20.00 and from 04.00 to 10.00), or 3 h in delay (LS, late sleep from 02.00 to 10.00, 0 lux, and dim light from 18.00 to 02.00). The fourth evening and night of each experimental week, subjects came to the laboratory at 18.00 and stayed in dim light from that moment until 00.00. After that they went to bed and were

instructed to sleep until they felt refreshed. The second week, six subjects started with ES and six with LS. The conditions were reversed in the fourth week.

### Subject characteristics

Subjects were recruited by advertisement in a newspaper. They were given information about the experiment and signed a written informed consent form. The 12 subjects of study 2 were matched for age and gender to the 12 subjects in study 1, with each group consisting of six females and six males. Three females and three males started in the second week with ML (study 1) or ES (study 2), the other three females and males started in the second week with EL (study 1) or LS (study 2). All subjects were in good physical and mental health. In study 1, age ranged from 23.6 to 56.5 y (mean  $\pm$  SD:  $39.3 \pm 12.1$ ). In study 2, age ranged from 21.5 to 56.7 y (mean  $\pm$  SD:  $38.2 \pm 12.8$ ). These values are not significantly different. The maximum age difference between matched subjects was 5.2 y. Subjects were not matched for the season they participated in. In eight of the 12 matched pairs, the experimental periods were at about the same time of the year (with a maximum difference of 3 months), in two pairs there were 4 months, and in two pairs 5 months between the experimental periods. It was not possible to avoid particular phases of the menstrual cycle in women, because of the 4-week duration of the experiment. We did not control the beginning of the experiment for menstrual phase.

### Measurements

Three physiological measures were recorded to estimate the phase of the circadian pacemaker and to analyse the effects on sleep: (i) melatonin concentration in saliva, (ii) core body temperature, and (iii) sleep EEG derived from C4-A1, C3-A2, EOG and EMG. On the fourth evening of each experimental week, the subjects were exposed to dim light between 18.00 and 00.00. During this time they remained seated, except for short walks. The subjects wore dark goggles during these walks. Bedtime and lights-off were scheduled at 00.00. As a consequence, the direct influences of environmental light, activity and time in bed or posture when assessing the circadian phase of the various variables were considered to be the same for all individuals in all conditions.

### Melatonin

On the fourth evening, saliva samples were collected hourly from 18.00 to 00.00. During this time, the subjects stayed in their rooms in dim light (< 10 lux). Subjects were sitting in a chair while producing the saliva to control for posture changes. No coffee or tea was served during this evening. The samples were frozen ( $-20^{\circ}\text{C}$ ) immediately, for later RIA analysis. The RIA procedure was essentially the same as that described by Vakkuri *et al.* (1984) and Vakkuri (1985), and was performed with [ $^3\text{H}$ ]-melatonin (2.93 Tbq/mmol, obtained from Amersham International, UK). However, in our procedure, the labelled

melatonin was added to the samples before the antiserum rather than simultaneously (antiserum obtained from Guildhay Antisera, Guildford, UK). The intra-assay coefficient of variation was found to be 14.3% ( $n=12$ ) at low concentrations (21 pM) and 6.8% ( $n=7$ ) at medium concentrations (44 pM). The interassay variation at low concentrations was 20.8% ( $n=60$ ).

DLMO was defined as the time of interception of a sigmoid-shaped curve (fitted through the hourly melatonin concentrations) with a criterium-level (arbitrarily defined) of 30 pmol/L. For five subjects in study 1 and six subjects in study 2, it turned out to be necessary to adjust this level (10–40 pmol/L) to obtain paired data (more details concerning saliva collection, the melatonin assay and the calculation of DLMO in Gordijn *et al.* 1998). In five cases in study 1 and in two cases in study 2, it was not possible to determine DLMO in all three conditions.

### Body temperature

Core body temperature was recorded on the third and fourth nights and on the fourth day of each experimental week. An ambulatory recording device was used either a Vitalog (sampling rate 0.25 min) or a home-made three-channel recording system (JOBLOG, Bakker and Beersma 1991) set to a 1-min sampling rate. Subsequently, the average of each set of four 0.25-min samples of the Vitalog system was calculated, which resulted in a 1-min data interval. In the present analysis the body temperature curves in the fourth night (00.00–07.00) of weeks 1, 2 and 4 were expressed as deviations from the mean body temperature between 00.00 and 07.00 in the fourth night of week 1. The time of the midpoint between the first interception of decreasing body temperature with the mean and the last interception of increasing body temperature with the mean was calculated ('mid-crossing point') to obtain an estimate of the circadian phase of body temperature.

### Sleep

Sleep EEG recordings were made throughout the third and fourth nights of each experimental week. The digitized signals (sampling rate EEG: 128 Hz; EOG and EMG: 64 Hz) were replayed on screen for visual inspection and scored according to Rechtschaffen and Kales (1968). The following sleep parameters were calculated: (i) sleep latency, i.e. the time between lights off and sleep onset (the first 30 s of stage 2 or REM sleep); (ii) waking-up time, i.e. the end of the last half-min stage 2, 3, 4 or REM sleep; (iii) tendency for sleep termination, i.e. the accumulation of sleep stages 0, 1 and 'movement time'; (iv) REM sleep latency, i.e. the time between sleep onset and the first 30 s of REM sleep; and (v) the duration of the first REM sleep episode.

In addition, the EEG signals were subjected to spectral analysis using fast Fourier transformation. Power densities between 0.5 and 30 Hz were calculated per 4-s periods, yielding data per 0.25 Hz. Data reduction was achieved by adding

power densities per min for the 0.5 Hz frequency and the 0.75–1 Hz frequency bin, and per 1 Hz bin width between 1.25 and 30 Hz. Slow wave activity (SWA), defined as the total power density in the 0.5–4 Hz frequency bins, was calculated during non-REM sleep (stages 2, 3 and 4).

### Statistics

Comparisons between two conditions were made using Wilcoxon matched pairs signed rank tests (two tailed).

## RESULTS

### Effects of light exposure and sleep displacement on circadian variables

#### Melatonin

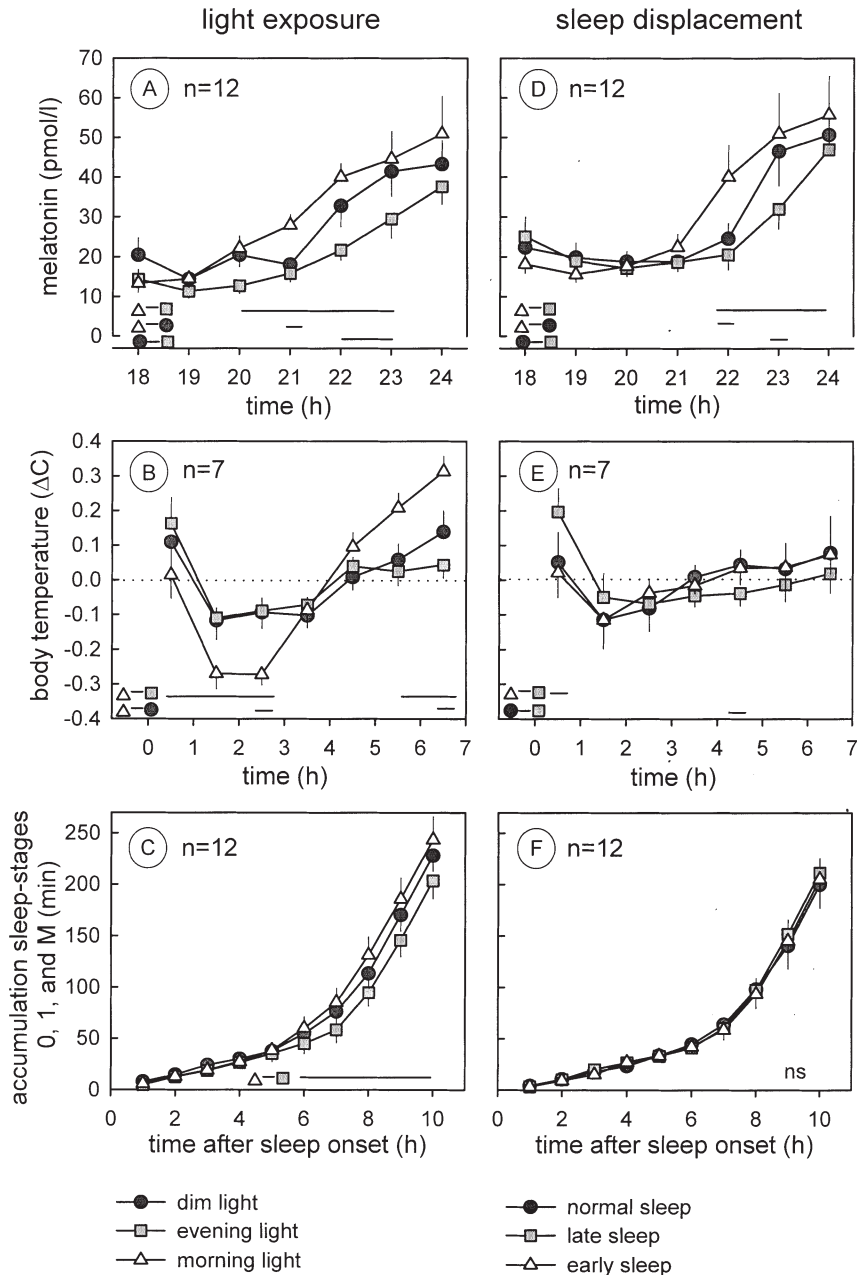
In each study, the average increases in melatonin levels on the fourth evening differed in the three conditions (Fig. 2a,d). In study 1 (Fig. 2a), the melatonin concentrations at 20.00, 21.00, 22.00 and 23.00 were significantly higher after ML than after EL. The melatonin concentrations after DL were higher at 22.00 and 23.00 than after EL, and lower at 21.00 than after ML. In study 2 (Fig. 2d), the melatonin concentrations at 22.00, 23.00 and 00.00 were significantly higher after ES than after LS. The melatonin concentration at 22.00 was significantly lower after NS than after ES, and at 23.00 it was significantly higher after NS than after LS.

These differences can be interpreted as phase shifts of the rising part of the melatonin curve. This is confirmed by the shifts in DLMO (Table 1). In study 1, DLMO in seven of nine subjects was earlier after ML than after EL (on average +89 min,  $P<0.05$ ). The timing of DLMO after ML and after DL (four of seven cases showed an earlier DLMO after ML than after DL) and after EL and DL (seven of eight cases showed a later DLMO after EL than after DL) was largely as expected but the average shifts were not significant (Table 1). In study 2, the timing of DLMO was phase advanced in nine of 10 subjects after ES compared with after LS (on average +95 min,  $P<0.01$ ) and phase delayed in 10 of 11 subjects after LS compared with after NS (on average –65 min,  $P<0.01$ ). In eight of 11 subjects the DLMO phase was advanced after ES compared with after NS, but the average shift was not significant (on average +28 min).

#### Body temperature

Some recordings of body temperature were missing or incomplete due to technical problems. In each of these studies, a group of seven subjects remained whose courses of body temperature in the fourth night could be compared for all three conditions. The average courses of body temperature during these nights are given in Figure. 2b,e. In study 1 (Fig. 2b), body temperature after ML was significantly lower during the first 3 h and higher during the last 2 h of the night when compared with body temperature after EL. In the third hour,





**Figure 2.** Results of three variables in study 1 (A–C) and study 2 (D–F). A/D, rise of melatonin concentration in saliva; B/E, course of body temperature, presented as a difference with respect to the average body temperature between 00.00 and 07.00 on the fourth night of the first week; and C/F, the accumulation of sleep stages 0, 1 and 'movement time (M)' on the fourth night

body temperature after ML was also lower than after DL and in the last hour body temperature was higher than after DL. There was no significant difference in the courses of body temperature between the EL and DL conditions.

The lower levels of body temperature after ML compared with those after EL in the first half of the night, and the higher levels of body temperature after ML compared with those after DL and EL in the second half of the night can be interpreted as a phase advance of the circadian rhythm of body temperature. The differences in the timing of the mid-crossings of body temperature with the mean support this interpretation.

The timing of the mid-crossing of body temperature was significantly earlier after ML than after DL (Table 1). There was a tendency for an earlier timing of body temperature after ML than after EL ( $P=0.07$ ). The timing of the mid-crossing of body temperature after EL was not significantly different from the timing after DL.

In study 2 (Fig. 2e), body temperature in the first hour of the fourth night after LS was significantly higher than body temperature in the first hour of the fourth night after ES. Body temperature in the fifth hour after LS was lower than body temperature in the fifth hour after NS. The timing of the

**Table 1** Phase shifts of DLMO, mid-crossing of body temperature and spontaneous sleep termination

Circadian variable	Study 1; light exposure		Study 2; sleep displacement			
	ML-DL	EL-DL	ML-EL	ES-NS	LS-NS	ES-LS
DLMO	+12 (23) <i>n</i> =7	−40 (32) <i>n</i> =8	+89 (31)* <i>n</i> =9	+28 (20) <i>n</i> =11	−65 (14)* <i>n</i> =11	+95 (27)* <i>n</i> =10
Body temperature	+50 (24)* <i>n</i> =9	−19 (16) <i>n</i> =8	+68 (29) <i>n</i> =8	−19 (32) <i>n</i> =9	−29 (15) <i>n</i> =8	+15 (21) <i>n</i> =8
Sleep termination	+21 (23) <i>n</i> =12	−18 (25) <i>n</i> =12	+39 (12)* <i>n</i> =12	+12 (23) <i>n</i> =12	+15 (25) <i>n</i> =12	−3 (12) <i>n</i> =12

The average shifts are expressed in minutes (SEM). A positive value represents a phase advance, a negative value a phase delay of the first condition relative to the second. For the explanation of the conditions see text. \* $P < 0.05$ .

mid-crossings of body temperature (Table 1) was not significantly different when the three conditions were compared in pairs, although there was a tendency for a later mid-crossing after LS compared with after NS ( $P = 0.09$ ).

### Sleep

On the fourth night, lights-off was scheduled at 00.00 in both studies. The actual times of lights-off were not different in the three conditions in either study (Table 2).

The 'tendency for sleep termination', represented by the accumulation of sleep stages 0, 1 and 'movement time' (Fig. 2c), speeds up earlier after ML than after EL, with the DL condition in between. At 6, 7, 8, 9 and 10 h after sleep onset the level of accumulated sleep stages 0, 1 and 'movement time' was significantly higher after EL than after ML. The results of the second study showed no significant differences in the accumulation of sleep stages 0, 1 and 'movement time' after NS, ES or LS (Fig. 2f). Another way of analysing these data is to compare the absolute times of spontaneous sleep termination (see Table 1 for the magnitude of the shifts between conditions and Table 2 for absolute sleep termination times). The average time of sleep termination after ML was significantly earlier than sleep termination after EL. The average time of sleep termination after DL lay in between, but was not significantly different from sleep termination after ML or after EL. In study 2, the absolute times of sleep termination were not significantly different in the three conditions.

In contrast to sleep termination, sleep latencies differed in the three conditions of the second study, but not in those of the first study (Table 2). Sleep latency after ES was shorter than sleep latencies after NS and after LS. REM sleep latencies were not different in the conditions in either study (Table 2).

The third sleep parameter to show a significant difference in one study, but not in the other, was the duration of the first REM sleep episode (Table 2). After ML, the duration of the first REM sleep episode was longer than after EL. The duration of the first REM sleep episode after DL lay in between, but this was not significantly different from either the ML or EL

condition. The duration of the first REM sleep episodes in the three conditions of study 2 did not differ significantly.

The unexpected finding of shifts in DLMO after sleep displacement justified further analyses. We looked for possible accompanying changes that could explain our results.

### Effects of sleep displacement on sleep homeostasis and REM sleep

#### Slow wave activity (SWA) in the fourth night after sleep displacement

On the basis of the two-process model of sleep regulation (Daan *et al.* 1984) we calculated the expected changes in the level of process S at 00.00 of the fourth night, as a result of the shifted sleep-phase and the accompanying changes in the duration of prior wakefulness. After subjects had woken up at 04.00 on the third night of the ES condition, the level of process S was expected to be increased by 8.4% at 00.00 on the fourth night compared with the level after the NS condition. After subjects had woken up at 10.00 on the third night of the LS condition, the expected value of S was 9.9% lower at 00.00 of the fourth night compared with S at 00.00 after NS. The actual difference between the ES and the NS condition in the accumulated SWA in the first 180 min of non-REM sleep was −0.2% (0.2% less SWA after ES than after NS). The level of accumulated SWA was 3.2% higher after LS than after NS. These small changes were significantly different from those predicted by the model ( $P < 0.05$ ). The levels of accumulated SWA in the first 180 min of non-REM sleep after ES, NS and LS did not differ significantly.

#### REM sleep in the third (displaced) and fourth nights

As mentioned earlier, REM sleep latencies and the duration of the first REM sleep episodes did not differ for conditions in the fourth night after sleep displacement. It is hypothesized, however, that the total amount of REM sleep during the third night of the three conditions, i.e. the last night with sleep at

different circadian phases, would be different. Therefore, we analysed it together with the total amount of REM sleep during the fourth night.

On the third night of the ES condition, with sleep from 20.00 to 04.00, the total amount of REM sleep ( $\pm$ SD) was  $84 \pm 22$  min. This was significantly different from the total amount of REM sleep on the third night of the NS condition (sleep from 23.00 to 07.00), which was  $94.5 \pm 14$  min ( $P < 0.05$ ), and from REM sleep on the third night of the LS condition (sleep from 02.00 to 10.00), which was  $102.5 \pm 21$  min ( $P < 0.02$ ). There were no significant differences in the total amounts of REM sleep on the fourth night of each experimental week: ES,  $111 \pm 30$  min; NS,  $108 \pm 34.5$  min; LS,  $101 \pm 23.5$  min (NS).

## DISCUSSION

### Effects of light exposure, study 1

#### Melatonin

The advanced timing of DLMO after 3 days of morning light (ML) relative to DLMO after 3 days of evening light (EL) was highly significant. The shifts of DLMO after ML and after EL relative to DLMO after DL were not significant. These shifts may represent shifts of the circadian pacemaker. The direction and magnitude of the shifts corresponded to the expectations based on the phase response curve (PRC) for melatonin to light (Van Cauter and Sturis 1994). This PRC, however, is based on a single 3-h 5000 lux pulse of bright light. In our study, subjects were exposed to three consecutive 3-h 2500 lux light pulses. The similarity of the results suggests that one single bright-light pulse has approximately the same phase-shifting potential as three bright-light pulses of moderate intensity, applied one after the other.

The non-significant advance ( $+12$  min) of DLMO after ML relative to DLMO after DL was obviously smaller, although not significantly so (Mann–Whitney  $U$ -test,  $Z = -1.39$ ,  $P = 0.16$ ), than the significant advance of  $+54$  min in the study of Dijk *et al.* (1989). Both age and sex differences between the groups of the two studies and differences in times of year when they were performed might explain this difference. In Dijk *et al.*'s experiment, eight young males took part, whereas our experiment comprised six males and six females with ages ranging from 23 to 56 y. Moreover, Dijk *et al.*'s study took place in February and March, while ours was performed throughout the year.

#### Body temperature

Because of missing data, the information on body temperature should be considered with caution. In addition, the fact that we did not control the start of the experiment according to menstrual phase in the six women in either study could have caused some noise over all conditions. Nevertheless, the close resemblance between our temperature curves after ML and DL and the curves presented in Dijk *et al.* (1987) would seem to support the reliability of the results. In our study, the

advance ( $+50$  min) of the mid-crossing of body temperature in the ML condition relative to that in the DL condition was significant. Three days of EL exposure were obviously not enough to induce a significant phase delay in body temperature. The phase advance after ML and the phase delay after EL are smaller than the shifts expected on the basis of the PRC, as assessed after applying three successive 5-h bright light pulses (7000–12 000 lux; Jewett *et al.* 1994). This is obviously due to the differences in duration and intensity of light exposure between the two studies. In our study, both the phase advance after ML and the absence of a phase delay after EL are more in line with the shifts predicted by the first published PRC, derived from experiments with a single 3-h bright-light pulse of 5000–9000 lux (Minors *et al.* 1991). This is understandable because of the nonlinear relationship between light intensity and phase-shift effect (Kronauer 1990).

#### Sleep

Our finding that sleep terminated earlier after ML than after EL supports the hypothesis that sleep termination can be manipulated by light exposure. The shifts are in line with the assumption of the two-process model that sleep timing is regulated by a circadian process (Borbély 1982; Daan *et al.* 1984). There was, however, a discrepancy with the data of Dijk *et al.* (1989). The average 21-min difference between sleep termination after ML and after DL in our study was not significant, unlike the 54-min difference found by Dijk *et al.* ( $P < 0.05$ ). In a direct comparison the results of the two studies are not significantly different (Mann–Whitney  $U$ -test,  $Z = -1.16$ ,  $P = 0.25$ ).

In the two-process model of sleep regulation (Daan *et al.* 1984), the upper threshold controlling sleep initiation is assumed to be parallel to the lower threshold, controlling sleep termination. Hence, if the circadian pacemaker is shifted, not only a shift in sleep termination would be expected, but also a shift in sleep initiation. We cannot test this prediction, as the time when sleep initiation was allowed was fixed at 00.00. Therefore, sleep initiation was not completely spontaneous. Nevertheless, sleep latency might have been shorter after ML than after EL. The data are unclear with respect to this issue. Sleep latency was, on average, rather short in all conditions (between 11 and 14 min), and no differences between conditions were found.

REM sleep latency was not influenced by light exposure. However, the duration of the first REM sleep episode did change. It was longer after ML than after EL. This difference can be interpreted as the result of a phase advance of the circadian influence on REM sleep production. An alternative explanation might be that the lengthening of the first REM sleep episode was related to the differences between the courses of body temperature after ML and EL (Czeisler *et al.* 1980; Dijk and Czeisler 1995). In this context, it should be mentioned that Cajochen *et al.* (1997) reported a longer first REM sleep episode after administering melatonin, a finding that was also accompanied by shifts in core body temperature (Kräuchi *et al.* 1997) and in the accumulation of sleep stages 0, 1 and 'movement time'.



**Table 2** Average clock time (SEM, in min) of lights-off and waking-up time; sleep latency, REM sleep latency, and the duration of the first REM sleep episode (last three variables in minutes) in the fourth night in study 1 and study 2

	Study 1; light exposure (n=12)			Study 2; sleep displacement (n=12)		
	ML	DL	EL	ES	NS	LS
Lights-off	00.05 (2)	00.05 (1)	00.07 (1)	00.02 (1)	00.04 (1)	00.04 (1)
Sleep termination	07.28 (19.5) <sup>1</sup>	07.49 (21)	08.07 (19) <sup>2</sup>	07.55 (17)	08.07 (24.5)	07.52 (14.5)
Sleep latency	13.5 (1.5)	11 (1.5)	13 (2)	10 (1.5) <sup>3,4</sup>	15.5 (2) <sup>5</sup>	20.5 (3) <sup>5</sup>
REM sleep latency	63 (6.5)	62 (6.5)	61 (6.5)	61 (8)	68 (7)	63 (3)
Duration of first REM episode	27 (4) <sup>1</sup>	21 (4)	17.5 (3) <sup>2</sup>	18.5 (3)	12 (2.5)	16 (2.5)

For the explanation of the abbreviations see text.

Study 1: <sup>1</sup>Significantly different ( $P < 0.05$ ) from EL.

<sup>2</sup>Significantly different ( $P < 0.05$ ) from ML.

Study 2: <sup>3</sup>Significantly different ( $P < 0.05$ ) from NS.

<sup>4</sup>Significantly different ( $P < 0.05$ ) from LS.

<sup>5</sup>Significantly different ( $P < 0.05$ ) from ES.

### Conclusion from study 1

In summary, the effects of the different light conditions on DLMO, core body temperature, spontaneous sleep termination and duration of first REM sleep episode can be interpreted convincingly as the consequences of phase shifts of the circadian pacemaker. A simplified diagram of the pathways that are supposedly involved is presented in Figure 3. They are indicated by the arrows with the mark '1'.

### Effects of sleep displacement, study 2

#### Melatonin

Sleep displacement resulted in phase shifts of DLMO. Whether the observed shifts in DLMO were accompanied by comparable

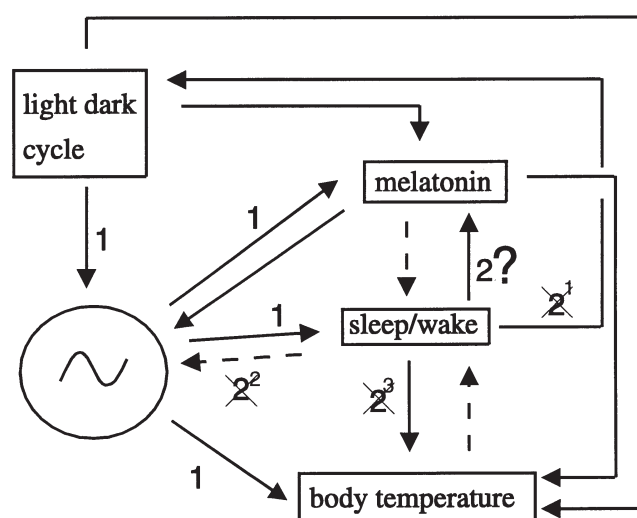
shifts in the acrophase and the offset of the melatonin rhythm is unknown. However, the changes in DLMO were large, and even of a similar magnitude to those observed in study 1. It is interesting to compare our results with those of Hoban *et al.* (1991). On average they did not find a significant shift in DLMO after a 2-h phase advance of sleep in six subjects, although the individual data showed some minor changes. Similarly, a 3-h phase advance of sleep in our 12 subjects did not result in a significant phase advance of DLMO. A 3-h phase delay of sleep did, however, cause a significant phase delay of DLMO. In addition, the difference in DLMO between the ES and LS condition (these conditions represent a 6-h difference in sleep phase) was also significant. Obviously, a 2- or 3-h phase advance of the sleep period is not enough to shift DLMO, whereas a 3-h delay and a 6-h difference in sleep-phase result in significant phase differences.

#### Body temperature

Neither advances in sleep-phase (ES) nor delays (LS) resulted in distinct changes in the courses of body temperature on the fourth night. The body temperature curves showed significant changes at two time points, but these changes cannot easily be explained by shifts in the entire curve of the body temperature rhythm. The average temperature curve after LS may suggest a somewhat slower rise than the curves after NS and ES, but the differences in the mid-crossing values did not reach significance.

#### Sleep

The curves of the accumulation of sleep stages 0, 1 and 'movement time' after the three instances of sleep displacement were not different either. This parallels the data on the times of spontaneous sleep termination: waking-up times after ES, NS and LS were not significantly different. Only the sleep latencies differed. This does not necessarily imply shifts of the upper threshold of process C. A more plausible explanation



**Figure 3.** Simplified, schematic presentation of the known and presumed interactions between the light-dark cycle, the circadian pacemaker and some overt rhythms in humans (for further explanation, see text).

for the small overall differences in sleep latency presents itself in the differences in the duration of prior wakefulness and the resulting sleep pressure between conditions. Finally, REM sleep latency and duration of the first REM sleep episode turned out to be unaffected by sleep displacement.

### General discussion

Below we explore three possible explanations for the unexpected shifts in DLMO after sleep displacement. The pathways which might be involved are shown in Fig. 3 (arrows with mark 2).

#### *The observed shifts in DLMO after sleep displacement reflect shifts of the circadian pacemaker*

This explanation seems unlikely for the following reasons. In study 1, the shifts in DLMO after bright-light exposure were accompanied by shifts in the course of body temperature and in the timing of sleep termination, as well as by a change in the duration of the first REM sleep episode. The consistency of these shifts provides a strong argument in favour of the interpretation of these shifts as being the result of light-induced shifts in the circadian pacemaker. In the same line, in study 2, the absence of clear shifts in body temperature and spontaneous sleep termination, together with the unaffected duration of the first REM sleep episode might be considered as evidence against involvement of the pacemaker underlying the shifts in DLMO. Normally, the rhythm of body temperature, sleep timing and the duration of the first REM sleep episode are more variable than the highly stable pattern of the melatonin rhythm. As a consequence the group size might have been too small to conclude that the variables were not significantly shifted (type II error). Since the magnitudes of the shifts in DLMO and the groups sizes were similar for the two experiments it remains remarkable that none of the three other variables in the second study were affected, while they were all affected in the first study.

In theory, there are two factors by which DLMO as an output variable of the circadian pacemaker could have been affected: (i) by a change in the light–dark cycle, or (ii) by a nonphotic effect of the shifted sleep–wake cycle. However, both possibilities seem unlikely as explained below.

(i) The shifted sleep–wake cycle caused changes in the perception of the light–dark cycle and this affected the circadian pacemaker (Fig. 3, arrow with mark ‘1’).

In all conditions we kept the subjects in dim light (< 10 lux) between 18.00 and 10.00. Therefore, it seems unlikely that the sleep displacement changed the LD cycles to such an extent that these changes could influence the circadian pacemaker. Obviously, there were very small differences between conditions in the exposure to light. Subjects were exposed to light with a maximum level of 10 lux from 04.00 to 07.00 in the ES condition, and to darkness (0 lux) in the LS condition. Conversely, subjects were exposed to a maximum level of 10 lux from 20.00 to 02.00 in the LS condition, but to darkness in the ES condition. Recent papers conclude that the circadian

pacemaker is affected by much lower light intensities than was previously thought (Boivin *et al.* 1996; Boivin and Czeisler 1998; Waterhouse *et al.* 1998). Light exposure of 180 lux during 5 h around the time of the body temperature minimum produced phase-advance shifts in both the body temperature and the melatonin rhythm of about 70 min (Boivin *et al.* 1996; Boivin and Czeisler 1998). Exposure to light with an intensity of 10–15 lux at the same phase resulted in phase delays of a similar magnitude as exposure to 0.03 lux light in both the body temperature (about –40 to –50 min, respectively) and the melatonin rhythm (about –45 to –90 min, respectively). Although from these data it has been concluded that the pacemaker can be shifted by ordinary room light, these results do not explain the phase shifts observed in our experiment where light exposure around the time of the body temperature minimum never exceeded 10 lux.

In our study, however, a contrast in light-intensity exposure (< 10 lux during waking, darkness during sleep) occurred in the night, whereas in the study of Boivin and Czeisler (1998) the background light intensity in the night was 10–15 lux and the sleep phase was scheduled during the subjective day. Only if the system is capable of detecting the difference of 10 lux light intensity during the night in our study, can it be supposed that the shift of the dark period within the dim light period caused the phase shifts observed in DLMO.

Our data are qualitatively consistent with the data of Van Cauter *et al.* (1998). They reported an acute 2-h phase advance of the onset and an almost 1-h advance of the offset of the melatonin rhythm after an 8-h advance of the sleep–wake cycle. Light intensity in their study changed from darkness during sleep to 200 lux during waking. They concluded that exposure to darkness in the afternoon affects human circadian phase. The shifts are reported only in the rhythm of melatonin levels. In the context of our results, it would be very interesting to know whether other circadian variables were shifted as well.

Finally, the subjects’ exposure to light in the daytime (between 10.00 and 18.00) was not controlled in our experiment. Until recently it was thought that light exposure during the daytime would not affect the circadian pacemaker, but light exposure shortly prior to and after the minimum of body temperature would. Therefore, we conducted our experiment in such a way that we carefully controlled light exposure from 18.00 to 10.00. Jewett *et al.* (1997) recently concluded that ‘the human circadian pacemaker is sensitive to light throughout the subjective day’. They reported phase shifts to 5 h of 10 000 lux bright-light exposure. Small phase advances were observed in response to light exposure in the morning, which seem to reduce linearly as a function of time of day to become phase delays in the evening. Therefore, they concluded that there is no ‘dead zone’ as is the case in a conventional phase–response curve. It is reasonable to suppose that our subjects were exposed to bright light during daytime, but not to 5 h of 10 000 lux. In addition, it seems unlikely that a sufficient number of subjects changed their habits of going outdoors in such a way that this could account for the observed differences in phase position of DLMO between conditions.

(ii) The shifted sleep–wake cycle acted as a nonphotic zeitgeber and caused direct phase shifts of the circadian pacemaker (Fig. 3, mark <sup>22</sup>).

At present, light is considered to be the main zeitgeber in humans. Although phase delays have been reported in circadian rhythms upon activity pulses (Van Reeth *et al.* 1994; Buxton *et al.* 1997), the issue of whether activity can act as a zeitgeber for the pacemaker in humans has not yet been settled (Redlin and Mrosovsky 1997). Some evidence for nonphotic entrainment has been reported recently in a blind individual (Klerman *et al.* 1998). In this study both body temperature and the melatonin rhythm seem to respond with a phase advance to a forced 23.8 h sleep–wake cycle. The authors concluded that ‘slow, gradual, and relatively small phase shifts may be all that nonphotic stimuli can elicit’ and probably only ‘in some individuals lacking ocular circadian photoreception’. The phase shifts of DLMO after our sleep displacements in our sighted individuals were as large as the shifts observed after light exposure and were observed immediately after the three manipulated nights. The nonphotic effects of the size demonstrated by Klerman *et al.* (1998) are insufficient to explain these observations. Beersma and Hiddinga (1998) subjected two age-matched groups of young males to high and low motor activity with a periodicity of 20 h. No effects on the circadian pacemaker were observed. Therefore, it seems unlikely that the 6-h difference in sleep phase in our experiment could have caused shifts of the circadian pacemaker by, for instance, the difference in the motor activity cycle.

The possibility of a nonphotic effect of sleep-timing on the circadian pacemaker in humans was discussed in two other papers. In the first (Japanese) paper, Nakamura (1996) published the results of three experiments. The author concluded that a forced 24-h sleep–wake schedule under circumstances of temporal isolation entrained the circadian rhythms of melatonin secretion (peak phase) and body temperature in three of eight subjects. However, the forced sleep–wake rhythm in this design closely resembles the endogenous period of the pacemaker. With this method it is very difficult to distinguish ‘entrainment’ from the individual’s endogenous period. Even if these findings could be replicated in a study with a forced sleep–wake schedule different from 24 h, the involvement of the circadian pacemaker would not be proven. The observation of a shifted melatonin rhythm might as well be explained by the shifted forced waking-up time itself, as observed in our study. This conclusion would contrast with the author’s interpretation of a fixed sleep–wake schedule acting as a nonphotic zeitgeber for the circadian pacemaker. Our reasoning is corroborated by Shanahan and Czeisler (1991) in a forced desynchrony protocol. They reported that in a subject living in temporal isolation, the rhythms of melatonin secretion and body temperature maintained a fixed phase relationship despite a 28-h sleep–wake schedule. This is an argument against the dependence of the melatonin rhythm on sleep phase.

The second paper concluding that there is a nonphotic effect of sleep timing on the circadian pacemaker was published by

Samková *et al.* (1997). In this study, where subjects stayed in low-intensity light for 4 days, the phase advanced position of the melatonin rhythm, achieved by one bright-light pulse in the morning, was maintained by having the subjects wake up at a fixed time early in the morning. Apart from melatonin secretion, no other circadian variables were measured. Therefore, it is unclear whether the melatonin rhythm is the only variable affected. In view of our data, an alternative explanation of Samková *et al.*’s data might be that the changed sleep phase directly caused the earlier timing of the melatonin rhythm, rather than affecting the pacemaker.

If we accept the conclusion that, in our study, the involvement of the circadian pacemaker in the phase-shifting effects of sleep displacement on DLMO is highly improbable, the question arises: what other factor could be responsible? Various uncontrolled differences could have occurred during the shifted sleep–wake cycles during daytime hours, for instance the timing of coffee consumption, eating snacks, daily pursuits, etc. In the present experiment we are not able to analyse the effects of these variables. We limited our search for an explanation to some variables that we can analyse.

#### **The observed shifts in DLMO after sleep displacement are due to changes in body temperature**

We are not aware of any study reporting direct effects of body temperature on DLMO. All studies on the interaction between melatonin levels and body temperature describe a diminishing effect of melatonin on body temperature and do not suggest a feedback mechanism in which body temperature affects melatonin secretion (e.g. Kräuchi *et al.* 1997). In addition, in the present study, only small effects on body temperature were found, whereas the DLMO shifts are relatively large. Therefore, it seems unlikely that the sleep displacement shifted DLMO via the effects on body temperature (Fig. 3, mark <sup>33</sup>).

#### **The observed shifts in DLMO after sleep displacement are related to a factor in sleep itself**

In our opinion, the possibility exists that a factor related to sleep itself affected the start of melatonin secretion (Fig. 3, mark <sup>22</sup>). This effect is not achieved by the circadian pacemaker or by changes in body temperature. For this reason we searched for changes in sleep that might correlate with the shifts. We discuss the possible involvement of process S or REM sleep below.

First, we hypothesized that the increase in sleep pressure resulting from the increase in the duration of wakefulness prior to the assessment of DLMO would affect the rising part of the melatonin rhythm. The subjects’ forced awakenings at 04.00 (ES), 07.00 (NS) and 10.00 (LS) at the end of the third displaced sleep period were expected to result in changes in the level of process S at 00.00. According to the two-process model of sleep regulation (Borbély 1982; Daan *et al.* 1984), S is reflected in the power density of the low-frequency bands (SWA) in the

sleep EEG. For this reason we assessed SWA in the fourth night, during the first 180 min of non-REM sleep. This method has been shown to be rather insensitive to (experimentally induced) changes in REM sleep (Beersma and Achermann 1995). Contrary to our expectation, the values of SWA after the ES and LS conditions did not differ from the value after the NS condition. These data differ significantly from those predicted by the model. Moreover, no significant difference was found between SWA after the ES and LS conditions, despite the 6-h difference in the duration of wakefulness these conditions bring with them. Consequently, the shifts in DLMO do not show any relationship to SWA.

Looking for explanations of both the shifted DLMO and the absence of SWA changes after sleep displacement, we hypothesized that the common cause might be a higher pressure for REM sleep after three nights in the ES condition and a smaller pressure for REM sleep after three nights in the LS condition. The proposed change in the pressure for REM sleep is due to the prevention (ES) or lengthening (LS) of sleep in the early morning hours in the sleep-displaced nights, a time at which the circadian propensity of REM sleep is high (Dijk and Czeisler 1995). Two studies (Beersma *et al.* 1990; Brunner *et al.* 1993) reported that an increase in the pressure for REM sleep induces a suppression of SWA in non-REM sleep. This might explain the absence of an increase in SWA after ES. Conversely, the absence of a decrease of SWA after LS could probably be explained by a reduction in the pressure for REM sleep. The assessment of the amount of REM sleep on the third night did reveal a significantly smaller amount of REM sleep in the ES condition than in the NS condition, and also a smaller amount in the ES condition than in the LS condition. The difference between the amounts in the ES and NS conditions is, however, only 10.5 min, and between those in ES and LS only 18.5 min. It is conceivable that the third night, with displaced sleep in the ES condition, already compensated for the REM sleep deficit of the first two nights, and therefore no REM sleep rebound was observed in the fourth night. Therefore, we cannot exclude the possibility that an increase in the pressure for REM sleep triggered an earlier increase of melatonin. An 18.5-min deficit of REM sleep accounts for ~ 17% of the normal amount of REM sleep. To compensate for this deficit, subjects need ~ 1.5 h more sleep. When we elaborate on this speculation, a shift of DLMO of ~ 1.5 h might trigger an earlier sleep initiation and in this way open the opportunity for a rebound of REM sleep.

### Final remark

The experimental conditions in this experiment are not common in normal sleep in everyday life. However, 6 more hours of wakefulness can easily occur in sleep-disordered patients. Our results raise doubts on the reliability of DLMO as a marker of circadian phase, especially in cases of sleep disturbances. Future experiments, in which the effects of sleep curtailment

or REM sleep deprivation in relation to melatonin secretion will be assessed may help to clarify our puzzling results.

### ACKNOWLEDGEMENTS

The authors thank Marjan Bleumink, Janine Engels, and Jaap Jansen for their help in data collection. Frans Flentge and Rikje Medema set up and performed the radioimmunoassay for melatonin in our laboratory. We are grateful to J.S. Borger for checking the English of the manuscript. This study was supported by N.W.O. grant 900-548-085 and completed with financial support of VWS grant 92-68.

### REFERENCES

- Arendt, J. Melatonin. *Clin. Endocrinol.*, 1988, 29: 205-229.
- Bakker, J. O. and Beersma, D. G. M. A digital 3-channel recorder for long term registrations. *Sleep Res.*, 1991, 20A: 485.
- Beersma, D. G. M. and Achermann, P. Changes of sleep EEG slow-wave activity in response to sleep manipulations: to what extent are they related to changes in REM sleep latency? *J. Sleep Res.*, 1995, 4: 23-29.
- Beersma, D. G. M., Dijk, D. J., Blok, C. G. H. and Everhardus, I. REM sleep deprivation during 5 hours leads to an immediate REM sleep rebound and to suppression of non-REM sleep intensity. *Electroencephalogr. Clin. Neurophysiol.*, 1990, 76: 114-122.
- Beersma, D. G. M. and Hiddinga, A. E. No impact of physical activity on the period of the circadian pacemaker in humans. *Chronobiol. Int.*, 1998, 15: 49-57.
- Boivin, D. B. and Czeisler, C. A. Resetting of circadian melatonin and cortisol rhythms in humans by ordinary room light. *Neuroreport*, 1998, 9: 779-782.
- Boivin, D. B., Duffy, J. F., Kronauer, R. E. and Czeisler, C. A. Dose-response relationships for resetting of human circadian clock by light. *Nature*, 1996, 379: 540-542.
- Borbély, A. A. A two process model of sleep regulation. *Human Neurobiol.*, 1982, 1: 195-204.
- Brunner, D. P., Dijk, D. J. and Borbély, A. A. Repeated partial sleep deprivation progressively changes the EEG during sleep and wakefulness. *Sleep*, 1993, 16: 100-113.
- Buxton, O. M., Frank, S. A., L'Hermite-Balériaux, M., Leproult, R., Turek, F. W. and Van Cauter, E. Roles of intensity and duration of nocturnal exercise in causing phase delays of human circadian rhythms. *Am. J. Physiol.*, 1997, 273: E536-E542.
- Cajochen, C., Kräuchi, K., Möri, D., Graw, P. and Wirz-Justice, A. Melatonin and S-20098 increase REM sleep and wake-up propensity without modifying NREM sleep homeostasis. *Am. J. Physiol.*, 1997, 272: R1189-R1196.
- Carr, D. B., Reppert, S. M., Bullen, B., Skrinar, G., Beitins, I., Arnold, M., Rosenblatt, M., Martin, J. B. and McArthur, J. W. Plasma melatonin increases during exercise in women. *J. Clin. Endocrinol. Metab.*, 1981, 53: 224-225.
- Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C. and Knauer, R. S. Human sleep: its duration and organization depends on its circadian phase. *Science*, 1980, 210: 1264-1267.
- Czeisler, C. A., Zimmerman, J. C., Ronda, J. M., Moore-Ede, M. C. and Weitzman, E. D. Timing of REM sleep is coupled to the circadian rhythm of body temperature in man. *Sleep*, 1980, 2: 329-346.
- Daan, S., Beersma, D. G. M. and Borbély, A. A. Timing of human sleep: recovery process gated by a circadian pacemaker. *Am. J. Physiol.*, 1984, 246: 161-183.
- Deacon, S. and Arendt, J. Posture influences melatonin concentrations in plasma and saliva in humans. *Neurosci. Lett.*, 1994, 167: 191-194.



- Dijk, D. J., Beersma, D. G. M., Daan, S. and Lewy, A. J. Bright morning light advances the human circadian system without affecting NREM sleep homeostasis. *Am. J. Physiol.*, 1989, 256: 106–111.
- Dijk, D. J. and Czeisler, C. A. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. *J. Neurosci.*, 1995, 15: 3526–3538.
- Dijk, D. J., Visscher, C. A., Bloem, G. M., Beersma, D. G. M. and Daan, S. Reduction of human sleep duration after bright light exposure in the morning. *Neurosci. Lett.*, 1987, 73: 181–186.
- Gordijn, M. C. M., Beersma, D. G. M., Korte, H. J. and Van den Hoofdakker, R. H. Testing the hypothesis of a circadian phase disturbance underlying depressive mood in non-seasonal depression. *J. Biol. Rhythms*, 1998, 13: 132–147.
- Gordijn, M. C. M., Jansen, J. H. C., Medema, H. M., Flentge, F. and Beersma, D. G. M. Melatonin in saliva, effect of toothpaste, coffee, smoking and lipstick. *Sleep Res.*, 1991, 20A: 493.
- Hoban, T. M., Lewy, A. J., Sack, R. L. and Singer, C. M. The effects of shifting sleep two hours within a fixed photoperiod. *J. Neural Transm. Gen. Sect.*, 1991, 85: 61–68.
- Honma, K. and Honma, S. A human phase response curve for bright light pulses. *Jpn. J. Psychiatry Neurol.*, 1988, 42: 167–168.
- Jewett, M. E., Kronauer, R. E. and Czeisler, C. A. Phase-amplitude resetting of the human circadian pacemaker via bright light: a further analysis. *J. Biol. Rhythms*, 1994, 9: 295–314.
- Jewett, M. E., Rimmer, D. W., Duffy, J. F., Klerman, E. B., Kronauer, R. E. and Czeisler, C. A. Human circadian pacemaker is sensitive to light throughout subjective day without evidence of transients. *Am. J. Physiol.*, 1997, 273: R1800–R1809.
- Klerman, E. B., Rimmer, D. W., Dijk, D. J., Kronauer, R. E., Rizzo, J. F., III and Czeisler, C. A. Nonphotic entrainment of the human circadian pacemaker. *Am. J. Physiol.*, 1998, 274: R991–R996.
- Kräuchi, K., Cajochen, C., Möri, D., Graw, P. and Wirz-Justice, A. Early evening melatonin and S-20098 advance circadian phase and nocturnal regulation of core body temperature. *Am. J. Physiol.*, 1997, 272: R1178–R1188.
- Kronauer, R. E. A quantitative model for the effects of light on the amplitude and phase of the deep circadian pacemaker, based on human data. In: J. Horne (Ed.) *Sleep '90*. Pontenagel Press, Bochum, 1990: 306–309.
- Lewy, A. J. and Sack, R. L. Phase typing and bright light therapy of chronobiologic sleep and mood disorders. In: A. Halaris (Ed.) *Chronobiology and Psychiatric Disorders*. Elsevier, New York, 1987: 181–205.
- Lewy, A. J., Sack, R. L. and Singer, C. M. Immediate and delayed effects of bright light on human melatonin production: shifting 'dawn' and 'dusk' shifts the Dim Light Melatonin Onset (DLMO). *Ann. NY Acad. Sci.*, 1985, 453: 253–259.
- Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A. and Markey, S. P. Light suppresses melatonin secretion in humans. *Science*, 1980, 210: 1267–1269.
- Minors, D. S., Waterhouse, J. M. and Wirz-Justice, A. A human phase-response curve to light. *Neurosci. Lett.*, 1991, 133: 36–40.
- Monteleone, P., Maj, M., Fusco, M., Orazzo, C. and Kemali, D. Physical exercise at night blunts the nocturnal increase of plasma melatonin levels in healthy humans. *Life Sci.*, 1990, 47: 1989–1995.
- Nakamura, K. Non-photic entrainment of human circadian clock – effects of forced sleep–wake schedule on the circadian rhythm in plasma melatonin. *Hokkaido J. Med. Sci.*, 1996, 71: 403–422.
- Rechtschaffen, A. and Kales, A. *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects*. BIS/BRI, Los Angeles, CA, 1968.
- Redlin, U. and Mrosovsky, N. Exercise and human circadian rhythms: what we know and what we need to know. *Chronobiol. Int.*, 1997 14: 221–229.
- Reiter, R. J. and Richardson, B. A. Some perturbations that disturb the circadian melatonin rhythm. *Chronobiol. Int.*, 1992, 9: 314–321.
- Samková, L., Vondrasová, D., Hájek, I. and Illnerová, H. A fixed morning awakening coupled with a low intensity light maintains a phase advance of the human circadian system. *Neurosci. Lett.*, 1997, 224: 21–24.
- Shanahan, T. L. and Czeisler, C. A. Intrinsic period of the endogenous circadian rhythm of plasma melatonin is consistent with that of core body temperature during forced desynchrony in a 21-year-old man. *Sleep Res.*, 1991, 20A: 557.
- Terman, M. Task force report on light treatment for sleep disorders. *J. Biol. Rhythms*, 1995, 10.
- Trinder, J., Armstrong, S. M., O'Brien, C., Luke, D. and Martin, M. J. Inhibition of melatonin secretion onset by low levels of illumination. *J. Sleep Res.*, 1996, 5: 77–82.
- Vakkuri, O. Diurnal rhythm of melatonin in human saliva. *Acta Physiol. Scand.*, 1985, 124: 409–412.
- Vakkuri, O., Leppäluoto, J. and Vuolteenaho, O. Development and validation of a melatonin radioimmunoassay using radioiodinated melatonin as tracer. *Acta Endocrinologica*, 1984, 106: 152–157.
- Van Cauter, E., Moreno Reyes, R., Akseki, E. L., 'Hermite-Balériaux, M., Hirschfeld, U., Leproult, R. and Copinschi, G. Rapid phase advance of the 24-h melatonin profile in response to afternoon dark exposure. *Am. J. Physiol.*, 1998, 275: E48–E54.
- Van Cauter, E. and Sturis, J. Demonstration of rapid light-induced advances and delays of the human circadian clock using hormonal phase markers. *Am. J. Physiol.*, 1994, 266: e953–e963.
- Van den Hoofdakker, R. H. Chronobiological theories of nonseasonal affective disorders and their implications for treatment. *J. Biol. Rhythms*, 1994, 9: 157–183.
- Van Reeth, O., Sturis, J., Byrne, M. M., Blackman, J. D., L'Hermite-Balériaux, M., Leproult, R., Olinier, C., Refetoff, S., Turek, F. W. and Van Cauter, E. Nocturnal exercise phase delays circadian rhythms of melatonin and thyrotropin secretion in normal men. *Am. J. Physiol.*, 1994, 266: E964–E974.
- Waterhouse, J., Minors, D., Folkard, S., Owens, D., Atkinson, G., Macdonald, I., Reilly, T., Sytnik, N. and Tucker, P. Light of domestic intensity produces phase shifts of the circadian oscillator in humans. *Neurosci. Lett.*, 1998, 245: 97–100.
- Wirz-Justice, A. Biological rhythms in mood disorders. In: F.E. Bloom and D.J. Kupfer (Eds) *Psychopharmacology: the Fourth Generation of Progress*. Raven, New York, 1995: 999–1017.